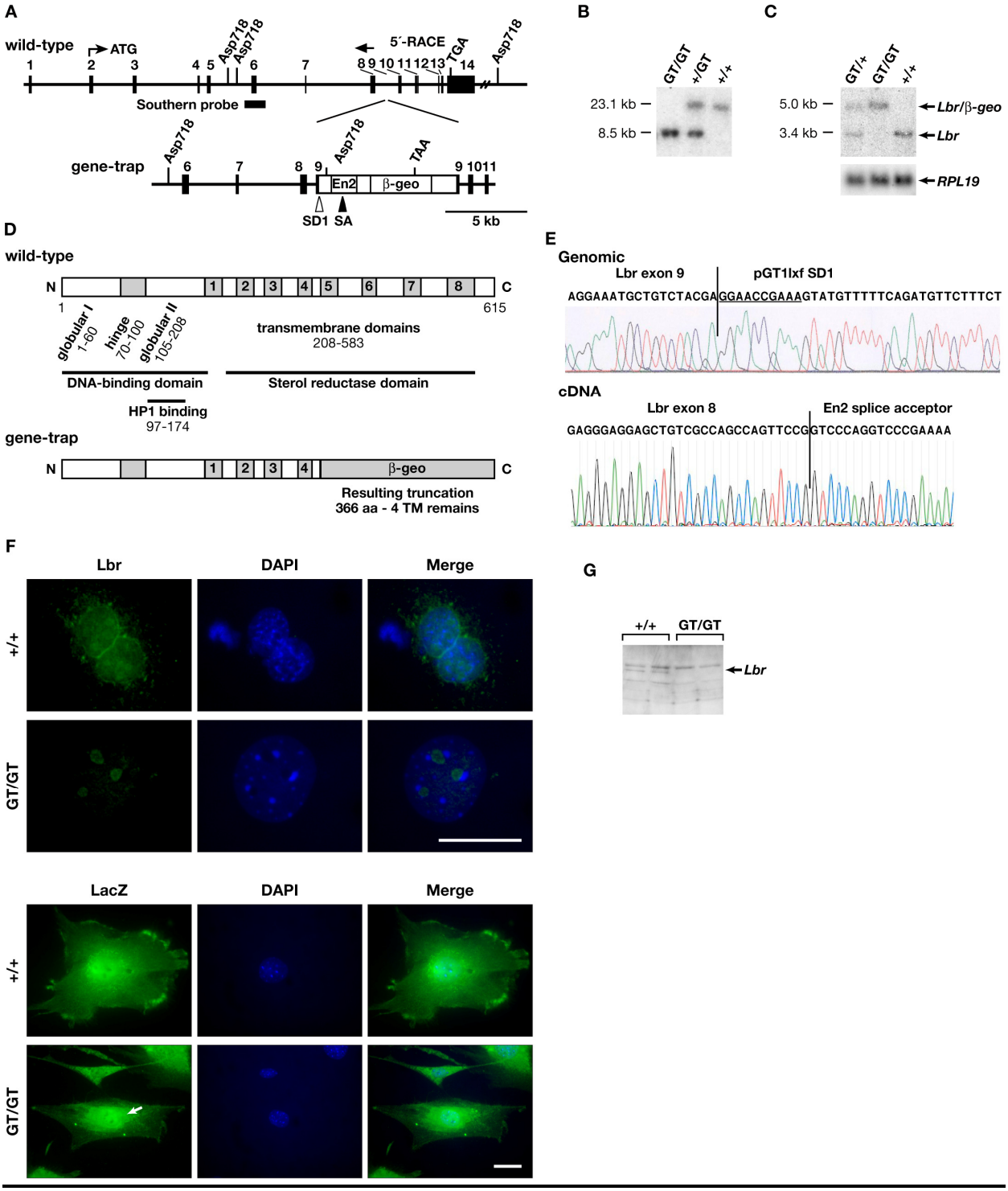
Supplemental Figure 1. The gene-trap insertion into the LBR locus

The *Lbr* gene consists of 14 exons indicated by the filled rectangles. Translation (ATG) begins at exon 2 and the stop codon is at exon 14 (TGA). The probe for the Southern analysis was to the region of exon 6. *Asp718* restriction enzyme sites used for Southern analysis are indicated. The 5'-RACE product is for sequence upstream of exon 8. In the gene-trapped allele, the gene-trap cassette has integrated within exon 9. A cryptic donor splice site (SD1) within the gene-trap vector directs splicing to the splice acceptor (SA) within the *En2* exon to produce a transcript in frame with *β-geo*. B. Southern analysis on genomic DNA digested with *Asp718* from wild-type (+/+), heterozygous (GT/+) and homozygous (GT/GT) fibroblasts. Size markers are indicated on the right. C. Northern analysis of total RNA from fibroblasts shows the *Lbr/β-geo* transcript. D. The wild-type LBR protein consists of a nucleoplasmic N-terminus, 8 transmembrane domains which contain the sterol reductase domain and a shorter C-terminus. In the putative LBR-β-geo protein, the N-terminus portion and 4 transmembrane domains remain while the C-terminus is replaced by the β-geo sequence. E. Upper: genomic sequence analysis showing integration of gene-trap vector into exon 9. Lower: cDNA sequence analysis showing integration of *En2* splice acceptor following exon 8. F. Upper panels: immunolabeling with a polyclonal rabbit LBR antiserum shows localization to the nuclear periphery in wild-type (+/+) which is lost in homozygous (GT/GT) fibroblasts and appears to show some intranuclear localization. Lower panels: immunolabeling with a lacZ antibody shows signal in the nucleoplasm and ER. Dapi was used to label nuclei. Scale bar, 20μm. G. Western blot of whole cell extracts from wild-type or homozygous (GT/GT) fibroblasts immunoblotted with an LBR antiserum. The 58kDa band is absent in GT/GT lanes.


A
wild-type
ATG
Asp718
Asp718
5´-RACE
TGA
Asp718
1 2 3 4 5 6 7 8 9 10 11 12 13 14
Southern probe
gene-trap
Asp718
6 7 8 9
Asp718
TAA
9 10 11
En2
β-geo
SD1 SA
5 kb
B
GT/GT
+/GT
+/+
23.1 kb
8.5 kb
C
GT/+
GT/GT
+/+
5.0 kb
3.4 kb
Lbr/β-geo
Lbr
RPL19
D
wild-type
N
C
1
615
globular I
1-60
hinge
70-100
globular II
105-208
transmembrane domains
208-583
DNA-binding domain
Sterol reductase domain
HP1 binding
97-174
gene-trap
β-geo
Resulting truncation
366 aa - 4 TM remains
E
Genomic
Lbr exon 9
pGT1lxf SD1
AGGAAATGCTGTCTACGAGGAACCGAAAGTATGTTTTTCAGATGTTCTTTCT
cDNA
Lbr exon 8
En2 splice acceptor
GAGGGAGGAGCTGTCGCCAGCCAGTTCCGGTCCCAGGTCCCGAAAA
F
Lbr
DAPI
Merge
+/+
GT/GT
LacZ
DAPI
Merge
+/+
GT/GT
G
+/+
GT/GT
Lbr

SUPPLEMENTAL TABLES

Supplemental Table 1: Double-stranded oligonucleotides used in EMSA

"-1400 LBR U"	AGTTGTCTTTCTCCA**ACTGCGCAAC**ATCTTGCTCTTTGAG
"-1400 LBR L"	CTCAAAGAGCAAGATGTTGCGCAGTTGGAGAAAGACAACA
"-1800 LBR U"	ACATCTAATTGCAA**ATTATGCAAC**CATGAGATTTAGATAG
"-1800 LBR L"	CTATCTAAATCTCATGGTTGCATAATTTGCAATTAGATGT
"-900 LBR U"	AGAGCTGAAGCTTAAGGGA**TTTGCCAAAC**TTTACGTTTAG
"-900 LBR L"	CTAAACGTAAAGTTTGGCAAATCCCTTAAGCTTCAGCTCT

Supplemental Table 2: Primers used in real-time PCR

Gene	Forward	Reverse
Cebpε	5'-GAGCCGAGATAAAGCCAAACA-3'	5'-AGGCAGCTGGCGGAAGAT-3'
Cebpα	5'-AGTACCGACTGCGACGTGAAC-3'	5'-GACCTTCTGCTGAGTCTCCATAATG-3'
C/EBPβ	5'-CGCCCGCGCACCACGACTTCCTCT-3'	5'-CGTCGCTCAGCTTGTCCACCGTCTT-3'
Mmp9 (Gel B)	5'-GGACGACGTGGGCTACGT-3'	5'-CTGCACGGTTGAAGCAAAGA-3'
Lct	5'-CCTGCACACTTGGACTTGCTT-3'	5'-GACAGAAACATCACGTGGTTGTC'